AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listing, of claims in the application:

Listing of Claims:

Claim 1. (withdrawn) A bacterial expression system for the production of rhUG comprising a synthetic gene which codes for human UG, wherein the synthetic gene comprises Seq. ID. Nos. 1-4.

Claim 2. (withdrawn) The expression system of claim 1, wherein the synthetic gene further comprises Met-Ala-Ala at the N terminus of the synthetic gene.

Claim 3. (withdrawn) A bacterial expression system for production of rhUG comprising a human cDNA sequence which codes for human UG wherein the gene further comprises Met-Ala-Ala at the N-terminus of the sequence.

Claim 4. (withdrawn) The expression system of claim 3, wherein the expression system further comprises an approximately 2.8 kb par sequence.

Claim 5. (withdrawn) A method of producing a rhUG research seed bank comprising:

a. inoculating onto a growth medium at least one colony of a bacterial strain comprising a rhUG expression system;

b. incubating the inoculated growth medium until a stationary phase is reached;

c. adding glycerol to the inoculated growth medium;

d. freezing the culture in aliquot portions; and

e. storing the frozen aliquot portions at a temperature below about -50 C.

Claim 6. (withdrawn) The method of claim 5, wherein the inoculated growth medium is incubated until an optical density measured between 550 nm to 660 nm of about 0.8 AU to 1.5 AU is reached.

Claim 7. (withdrawn) The method of claim 5, wherein the cryopreservative comprises glycerol.

Claim 8. (withdrawn) The method of claim 5, wherein the aliquot portion is about 1 ml.

Claim 9. (withdrawn) The method of claim 5, wherein the storage temperature is between about -70 and about -90 °C.

Claim 10. (withdrawn) A method of producing a rhUG master cell bank comprising:

a. inoculating a suitable incubating broth with an aliquot portion of a rhUG research seed bank to form a bacterial culture;

b. incubating the bacterial culture;

c. adding a cryopreservative to the bacterial culture to form a cryopreserved solution;

d. transferring a portion of the cryopreserved solution to a cryovial; and

e. storing the cryovial at a temperature below about -60 C.

Claim 11. (withdrawn) The method of claim 10, wherein the culture is incubated until an optical density measured between 550 nm to 660 nm of about 0.8 AU to 1.5 AU reached.

Claim 12. (withdrawn) The method of claim 10, wherein the cryopreservative comprises glycerol.

Claim 13. (withdrawn) The method of claim 10, wherein the portion transferred to a cryovial is about 1 ml.

Claim 14. (withdrawn) The method of claim 10, wherein the storage temperature is between about -70 and about -90 C.

Claim 15. (withdrawn) A method of producing a rhUG production cell bank comprising:

a. inoculating a suitable incubating broth with an aliquot portion of a rhUG master cell bank to form a bacterial culture;

b. incubating the bacterial culture;

c. adding a cryopreservative to the bacterial culture to form a cryopreserved solution;

d. transferring a portion of the cryopreserved solution to a cryovial; and

e. storing the cryovial at a temperature below about -60 C.

Claim 16. (withdrawn) The method of claim 15, wherein the bacterial culture is incubated until an optical density measured between 550 nm to 660 nm of about 0.8 AU to 1.5 AU is reached.

Claim 17. (withdrawn) The method of claim 15, wherein the cryopreservative comprises glycerol.

Claim 18. (withdrawn) The method of claim 15, wherein the portion transferred to a cryovial is about 1 ml.

Claim 19. (withdrawn) The method of claim 15, wherein the storage temperature is between about -70 and about -90 C.

Claim 20. (withdrawn) A method of expressing rhUG comprising the steps of:

a. providing a production seed cell bank culture comprising an expression vector capable of expressing rhUG;

b. inoculating a broth medium with the production seed cell bank culture to form an inoculum;

c. incubating the bacterial culture formed in step b;

d. inoculating a large scale fermenter with the inoculum formed in step c to form a fermentation culture;

e. incubating the fermentation culture within the large scale fermenter;

f. adding an induction agent to the fermentation culture to induce the expression of rhUG; and

g. harvesting the fermentation culture after step f.

Claim 21. (withdrawn) The method of claim 20, wherein the expression vector comprises Seq. ID Nos. 1-4.

Claim 22. (withdrawn) The method of claim 20, wherein the inoculum is incubated for a period between about 12 hours and about 24 hours at a temperature between about 28 °C and about 36 °C.

Claim 23. (withdrawn) The method of claim 20, wherein the large scale fermenter has at least a 300 liter capacity.

Claim 24. (withdrawn) The method of claim 20, wherein the incubation of step e is continued until an optical density 550 nm to 660 nm until a minimum OD of 2.0 AU is reached.

Claim 25. (withdrawn) The method of claim 20, wherein the induction agent comprises isopropyl-beta-D-thiogalactopyranoside (IPTG).

Claim 26. (withdrawn) The method of claim 20, wherein of about 1 to about 4 hours elapses between step f and step g.

Claim 27. (withdrawn) The method of claim 20, wherein harvesting the fermentation culture comprises centrifugation.

Claim 28. (withdrawn) A method of expressing rhUG comprising the steps of:

a. inoculating a large scale fermenter with an inoculum comprising an expression vector capable of expressing rhUG to form a fermentation culture;

b. incubating the fermentation culture within the large scale fermenter;

c. adding an induction agent to the fermentation culture to induce the expression of rhUG; and

d. harvesting the fermentation culture.

Claim 29. (withdrawn) The method of claim 28, wherein the expression vector comprises Seq. ID Nos. 1-4.

Claim 30. (withdrawn) The method of claim 28, wherein the large scale fermenter has at least a 300 liter capacity.

Claim 31. (withdrawn) The method of claim 28, wherein the incubation of step b is continued until an optical density 550 nm to 660 nm until a minimum OD of 2.0 AU is reached.

Claim 32. (withdrawn) The method of claim 28, wherein the induction agent comprises isopropyl-beta-D-thiogalactopyranoside (IPTG).

Claim 33. (withdrawn) The method of claim 28, wherein of about 1 to about 4 hours elapses between step c and step d.

Claim 34. (withdrawn) The method of claim 28, wherein harvesting the fermentation culture comprises centrifugation.

Claim 35. (original) A method of purifying rhUG comprising the steps of:

a. providing a bacterial cell paste comprising bacterial cells capable of overexpressing rhUG;

b. lysing the bacterial cell paste to form a supernatant;

c. filtering the supernatant formed in step b through a first nominal molecular weight cut off (NMWCO) membrane to form a first permeate;

d. concentrating the first permeate formed in step c by the use of a second NMWCO membrane;

e. loading the concentrated permeate formed in step d onto an anion exchange column to form a first eluate;

f. concentrating the first eluate formed in step e by the use of a third NMWCO membrane to form a second concentrate;

g. loading the second concentrate formed in step f onto a Hydroxyapatite (HA) column to form a second eluate;

h. separating host-derived proteins from the rhUG in the second eluate formed in step g to provide purified rhUG; and

i. recovering the purified rhUG formed in step h.

Claim 36. (amended) The method of claim 35, wherein the synthetic gene expressed in the bacterial cells comprises at least one of a group comprising Seq. ID Nos. 1-4.

Claim 37. (original) The method of claim 35, wherein lysing comprises shearing.

Claim 38. (original) The method of claim 35, wherein between step b and step c, cell debris is removed by centrifugation.

Claim 39. (original) The method of claim 35, wherein the membrane of step b is about a 30K to 100K NMWCO membrane.

Claim 40. (original) The method of claim 39, wherein the filtering of step c comprises the use of a tangential flow filtration (TFF) system.

Claim 41. (original) The method of claim 35, wherein the membrane of step d is about a 5K NMWCO membrane.

Claim 42. (original) The method of claim 41, wherein the anion exchange column of step e is a Macro Q anion exchange column.

Claim 43. (amended) The method of claim 41, wherein the host-derived proteins of step h are separated with a Chelating ~~Sepharose~~ Fast Flow (CSFF) resin column.

Claim 44. (original) The method of claim 43, wherein the CSFF resin column comprises copper.

Claim 45. (original) The method of claim 44, wherein after step h a positively charged membrane is placed downstream of the CSFF column forming a pass through substantially free of host derived proteins.

Claim 46. (amended) The method of claim 45, wherein the positively charged membrane is a ~~Sartobind Q TFF~~ filtration membrane.

Claim 47. (original) The method of claim 35, wherein the second eluate is diafiltered through about a 30K NMWCO membrane.

Claim 48. (original) The method of claim 35, wherein the rhUG recovered in step i is substantially free of aggregates.

Claim 49. (amended) A method of purifying rhUG comprising the steps of:

a. providing bacterial cells capable of overexpressing rhUG;

b. lysing the bacterial cells to form a supernatant liquid;

c. filtering the liquid through a molecular weight cut off (NMWCO) membrane;

d. loading the liquid onto an ion exchange column;

e. separating host-derived proteins from the rhUG to provide purified rhUG; and

f. recovering the purified rhUG.

Claim 50. (amended) The method of claim 49, wherein the synthetic gene expressed in the bacterial cells comprises at least one of a group comprising Seq. ID Nos. 1-4.

Claim 51. (original) The method of claim 49, wherein the filtering of step c comprises the use of a tangential flow filtration (TFF) system.

Claim 52. (amended) The method of claim 49, wherein the ~~anion~~ ion exchange column of step d is a Macro Q anion exchange column.

Claim 53. (amended) The method of claim 49, wherein the host-derived proteins of step ~~h~~ e are separated with a Chelating ~~Sepharose~~ Fast Flow (CSFF) resin column.

Claim 54. (original) The method of claim 49, wherein the rhUG recovered in step ~~i~~ f is substantially free of aggregates.

Claim 55. (amended) A method of producing a pharmaceutical grade rhUG drug substance comprising the steps of:

a. providing a bacterial expression system capable of expressing rhUG;

b. inoculating a fermenter with an inoculum comprising the bacterial expression system to form a fermentation culture;

c. adding an induction agent to the fermentation culture to induce the expression of rhUG by the bacterial expression system;

d. harvesting the rhUG expressed in step c; and

e. purifying the rhUG harvested in step d, wherein the purifying step comprises the use of at least one filtration step and at least one ~~exhange~~ exchange column.

Claim 56. (original) An assay method for determining the potency of recombinant human uteroglobin in a sample which comprises:

(a) contacting a sample containing recombinant human uterogloblin with phospholipase A_2,

(b) introducing a labeled substrate to said sample,

(c) separating product from sample, and

(d) determining level of cleavage.

Claim 57. (withdrawn) The method of claim 56, wherein the assay is used in conjunction with a standard ^{14}C-labeled assay.

Claim 58. (withdrawn) The method of claim 56, wherein the radiolabeled substrate is 1-stearoyl-2-[^{14}C]arachidonyl phosphotidyl choline.

Claim 59. (withdrawn) The method of claim 56, wherein the recombinant human uteroglobin phospholipase A_2 is added to a final concentration of 2nM to 200nM.

Claim 60. (withdrawn) The method of claim 56, wherein the sample of step (a) is preincubated for 15 minutes to 30 minutes at 30 °C to 40 °C.

Claim 61. (withdrawn) The method of claim 56, wherein the reaction in step (b) is stopped by addition of an organic phase stopping solution.

Claim 62. (withdrawn) The method of claim 56, wherein the sample in step (c) is separated by vortexing and centrifugation.

Claim 63. (withdrawn) The method of claim 56, wherein the product of step (c) is separated from the sample by liquid-liquid separation.

Claim 64. (withdrawn) The method of claim 56, wherein the level of cleavage in step (d) is determined by scintillation counting.

Claim 65. (withdrawn) A method for measuring *in vitro* the anti-inflammatory effect arising from inhibition or blocking of secretory phopsholipase A_2 enzymes by recombinant human uteroglobin, comprising:

(a) contacting a sample containing recombinant human uterogloblin with phospholipase A_2,

(b) introducing labeled substrate to said sample,

(c) separating product from sample, and

(d) determining level of cleavage by scintillation counting.

Claim 66. (withdrawn) An assay method for assaying for the inhibition of secretory phopsholipase A_2 activity by recombinant human uteroglobin, comprising:

(a) contacting a sample containing recombinant human uterogloblin with phospholipase A_2,

(b) introducing labeled substrate to said sample,

(c) separating product from sample, and

(d) determining level of cleavage by scintillation counting.

Claim 67. (withdrawn) An assay method for determining the potency of recombinant human uteroglobin in a sample which comprises:

(a) contacting a sample containing recombinant human uterogloblin with phospholipase A_2,

(b) introducing flourescently labeled substrate to said sample,

(c) separating non-cleaved substrate from sample, and

(d) determining amount of cleaved substrate by flourescence.

Claim 68. (withdrawn) The method of claim 67, wherein the sample of recombinant human uteroglobin in step (a) has a final concentration of 34nM to 34μM.

Claim 69. (withdrawn) The method of claim 67, wherein the sample of step (a) is preincubated for about 15 to 30 minutes at about 30 to 40 °C.

Claim 70. (withdrawn) The method of claim 67, wherein the flourescently-labeled substrate is 2-decanoyl-1-(O-(11-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3 propionyl)amino)undecyl)-sn-glycero-3-phosphotidylcholine.

Claim 71. (withdrawn) The method of claim 67, wherein the reaction in step (b) is stopped by addition of an organic phase stopping solution.

Claim 72. (withdrawn) The method of claim 67, wherein in step (c) 1μL to 100 μL of the stopped assay is loaded directly onto a silica normal phase HPLC column.

Claim 73. (withdrawn) A method for measuring *in vitro* the binding of recombinant human uteroglobin to fibronectin, comprising:

(a) contacting a recombinant fragment of human fibronectin with a recombinant human CC10-HRP conjugate,

(b) visualizing the assay to determine binding of recombinant human uteroglobin to the fibronectin fragment.

Claim 74. (amended) The method of claim 35 further comprising steps for determining the purity of recombinant human uteroglobin comprising, ~~A method for determining the purity of recombinant human uteroglobin which comprises,~~

(a) taking samples of intermediates at each step within the process of claim 35 and

(b) analyzing the process intermediates to determine purity relative to unpurified recombinant human uteroglobin or

(c) analyzing the process intermediates to determine purity relative to purified recombinant human uteroglobin taken from the preceding step or steps of the process of claim 35.

Claim 75. (original) The method of claim 74, wherein process intermediates are analyzed by SDS-PAGE.

Claim 76. (original) The method of claim 74, wherein process intermediates are analyzed by rhUG ELISA.

Claim 77. (original) The method of claim 74, wherein process intermediates are analyzed by LAL.

Claim 78. (original) The method of claim 74, wherein process intermediates are analyzed for protein content.

Claim 79. (withdrawn) A pharmaceutical composition comprising the purified recombinant human uteroglobin of claim 35.

Claim 80. (withdrawn) A pharmaceutical composition comprising a purified recombinant human uteroglobin and a pharmaceutically acceptable carrier or diluent.

Claim 81. (withdrawn) The pharmaceutical composition of claim 80 wherein said recombinant human uteroglobin contains less than 5% aggregates of recombinant human uteroglobin.

Claim 82. (withdrawn) The pharmaceutical composition of claim 80 wherein said recombinant human uteroglobin has a purity of greater than 95%.

Claim 83. (withdrawn) The pharmaceutical composition of claim 80 wherein endotoxin levels within said recombinant human uteroglobin comprises less than 5 EU/mg rhUG.

Claim 84. (withdrawn) The pharmaceutical composition of claim 80 wherein said recombinant human uteroglobin is in a sodium chloride solution.

Claim 85. (withdrawn) The pharmaceutical composition of claim 80 wherein said recombinant human uteroglobin is stable in solution at 4 °C for at least 4 months.

Claim 86. (withdrawn) The pharmaceutical composition of claim 80 wherein said recombinant human uteroglobin is stable in solution at 4 °C for at least 6 months.

Claim 87. (withdrawn) The pharmaceutical composition of claim 80 wherein said recombinant human uteroglobin is stable in solution at 4°C for at least 9 months.

Claim 88. (withdrawn) The pharmaceutical composition of claim 80 wherein said recombinant human uteroglobin is stable in solution at 4 °C for at least 12 months.

Claim 89. (withdrawn) The pharmaceutical composition of claim 80 wherein said recombinant human uteroglobin is stable in solution at 4°C for at least 15 months.

Claim 90. (withdrawn) The pharmaceutical composition of claim 80 wherein said recombinant human uteroglobin is stable in solution at 4 °C for at least 18 months.

Claim 91. (withdrawn) The pharmaceutical composition of claim 80 wherein said recombinant human uteroglobin is stable is solution at 25 °C and 60% Room Humidity for at least 1 month.

Claim 92. (withdrawn) The pharmaceutical composition of claim 80 wherein said recombinant human uteroglobin is stable is solution at 25 °C and 60% Room Humidity for at least 2 months.

Claim 93. (withdrawn) The pharmaceutical composition of claim 80 wherein said recombinant human uteroglobin is stable is solution at 25 °C and 60% Room Humidity for at least 4 months.

Claim 94. (withdrawn) The pharmaceutical composition of claim 80 wherein said recombinant human uteroglobin is stable is solution at 25 °C and 60% Room Humidity for at least 7 months.

Claim 95. (withdrawn) The pharmaceutical composition of claim 80 wherein said recombinant human uteroglobin is safe to administer to a mammal.

Claim 96. (withdrawn) The pharmaceutical composition of claim 80 wherein said recombinant human uteroglobin is safe to administer to a human.

Claim 97. (withdrawn) The pharmaceutical composition of claim 80 wherein said recombinant human uteroglobin is safe to administer via an intratracheal tube.

Claim 98. (withdrawn) The pharmaceutical composition of claim 80 wherein said recombinant human uteroglobin is safe to administer to a premature infant.

Claim 99. (withdrawn) The pharmaceutical composition of claim 80 wherein said recombinant human uteroglobin is safe to administer to a patient receiving artificial surfactant.

Claim 100. (withdrawn) The pharmaceutical composition of claim 80 wherein said recombinant human uteroglobin is safe to administer to a patient in respiratory distress.